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Information in support of a Recovery Potential Assessment of Threehorn Wartyback (*Obliquaria reflexa*) in Canada

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

In May 2013, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assessed the status of Threehorn Wartyback (*Obliquaria reflexa*) and determined the designation to be Threatened. The reason provided for this designation is that, "This rare species historically occurred in the Great Lakes drainages including Lake St. Clair, western Lake Erie, and the Grand, Thames, and Detroit rivers. The species has not been found since 1992 in Lake St. Clair and the Detroit River and may be extirpated there due largely to the impacts of Zebra and Quagga mussels. It was last recorded from the Canadian side of Lake Erie in 1997. Pollution (sediment loading, nutrient loading, contaminants and toxic substances) related to both urban and agricultural activities represent a high and continuing threat at the three remaining riverine locations." Threehorn Wartyback is currently not listed under the *Species at Risk Act* (SARA).

This Recovery Potential Assessment (RPA) provides information and scientific advice needed to fulfill various requirements of the SARA. The information may be used to inform the development of recovery documents and for assessing permits, agreements and related conditions, as per section 73, 74, 75, 77 and 78 of the SARA. The scientific information also serves as advice to the Minister of Fisheries and Oceans Canada (DFO) regarding the listing of the species under the SARA and is used when analyzing the socio-economic impacts of adding the species to the list as well as during subsequent consultations, where applicable.

This Research Document describes the current state of knowledge of the biology, ecology, distribution, population trends, and habitat requirements of Threehorn Wartyback. Threehorn Wartyback population sensitivity to perturbations, as well as the threats currently effecting known Threehorn Wartyback populations is discussed. Mitigation measures and alternative activities related to the identified threats, which can be used to protect the species, are also presented. This assessment considers the available scientific data with which to assess the recovery potential of Threehorn Wartyback in Canada.

Information donnée à l'appui d'une évaluation du potentiel de rétablissement de l'obliquaire à trois cornes (*Obliquaria reflexa*) au Canada

RÉSUMÉ

En mai 2013, le Comité sur la situation des espèces en péril au Canada (COSEPAC) a évalué la situation de l'obliquaire à trois cornes (*Obliquaria reflexa*) et lui a attribué le statut d'espèce menacée. La justification de cette désignation est la suivante : « Cette espèce rare était historiquement présente dans les bassins hydrographiques des Grands Lacs, y compris le lac Sainte-Claire, l'ouest du lac Érié et les rivières Grand, Thames et Détroit. Elle n'a pas été trouvée depuis 1992 dans le lac Sainte-Claire et la rivière Détroit et pourrait y être disparue principalement en raison des impacts des moules zébrée et quagga. L'espèce a été observée pour la dernière fois du côté canadien du lac Érié en 1997. La pollution (charge sédimentaire, charge en éléments nutritifs, contaminants et substances toxiques) liée aux activités urbaines et agricoles représente une menace grave et continue dans les trois localités fluviales restantes ». À l'heure actuelle, l'obliquaire à trois cornes n'est pas inscrite sur la liste de la *Loi sur les espèces en péril* (LEP).

La présente évaluation du potentiel de rétablissement (EPR) fournit les renseignements et les avis scientifiques nécessaires pour satisfaire à diverses exigences de la LEP. Les renseignements peuvent servir de base à l'élaboration de documents relatifs au rétablissement et à l'évaluation des permis, des ententes et des conditions connexes, conformément aux articles 73, 74, 75, 77 et 78 de la LEP. On se sert également de ces renseignements scientifiques pour conseiller le ministre des Pêches et des Océans (MPO) au sujet de l'inscription de l'espèce en vertu de la LEP, analyser les répercussions socio-économiques de l'inscription de l'espèce sur la liste ainsi que pour les consultations subséquentes, le cas échéant.

Le présent document de recherche fournit une description de l'état actuel des connaissances de la biologie, de l'écologie, de la répartition, des tendances démographiques et des besoins en matière d'habitat de l'obliquaire à trois cornes. On y aborde la vulnérabilité des populations d'obliquaire à trois cornes aux perturbations, ainsi que les menaces touchant actuellement les populations connues. Des mesures d'atténuation et d'autres activités associées aux menaces déterminées, qui peuvent être utilisées dans le but de protéger l'espèce, sont également présentées. Cette évaluation tient compte de toutes les données scientifiques existantes permettant d'évaluer le potentiel de rétablissement de l'obliquaire à trois cornes au Canada.

SPECIES INFORMATION

Scientific Name – *Obliquaria reflexa* (Rafinesque, 1820)

Common Name – Threehorn Wartyback

Current COSEWIC Status (Year of Designation) – Threatened (May 2013)

Current *Species at Risk Act* Status (Schedule) – No status (No schedule)

Current Ontario *Endangered Species Act* Status – No status

Range in Canada – Ontario

BACKGROUND

Designation

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assessed the status of Threehorn Wartyback (*Obliquaria reflexa*) as Threatened. The reason given for this designation was that, "This rare species historically occurred in the Great Lakes drainages including Lake St. Clair, western Lake Erie, and the Grand, Thames, and Detroit rivers. The species has not been found since 1992 in Lake St. Clair and the Detroit River and may be extirpated there due largely to the impacts of Zebra and Quagga mussels. It was last recorded from the Canadian side of Lake Erie in 1997. Pollution (sediment loading, nutrient loading, contaminants and toxic substances) related to both urban and agricultural activities represent a high and continuing threat at the three remaining riverine locations." Threehorn Wartyback is currently not listed under the *Species at Risk Act* (SARA). A Recovery Potential Assessment (RPA) process has been developed by Fisheries and Oceans Canada (DFO) to provide information and scientific advice needed to fulfill SARA requirements, including the development of recovery strategies and authorizations to carry out activities that would otherwise violate the SARA (DFO 2007). This document provides background information on Threehorn Wartyback to inform the RPA.

Species Description

Threehorn Wartyback is a medium-sized freshwater mussel with an average shell length of approximately 40 mm (Metcalfe-Smith et al. 2005), while a maximum shell length of 80 mm has been reported from US waters (COSEWIC 2013). Lengths of Threehorn Wartyback recorded from the Sydenham River (n=37) ranged from 15 to 64 mm, while lengths recorded from the Thames River (n=24) ranged from 28 to 62 mm (Figure 1). Of the shells available from the Grand River (n=64), sizes ranged from 23.4 to 56 mm (Figure 1). In a study on the variations of reproductive traits, Haag and Staton (2003) noted that male Threehorn Wartyback collected from the Little Tallahatchie River (Mississippi, United States) were significantly larger than female Threehorn Wartyback; however, this information is not currently available from Canadian populations.

The shell is described as thick, circular to triangular, and inflated (Figure 2; Metcalfe-Smith et al. 2005). The anterior end is rounded, and the posterior end is bluntly pointed (Metcalfe-Smith et al. 2005). While Threehorn Wartyback is dioecious, they lack pronounced sexual dimorphism (COSEWIC 2013). The most prominent shell feature is the single row of two to five knobs extending from the beak to the ventral margin, which alternate in position between valves (Metcalfe-Smith et al. 2005). The beaks are elevated and curved inward (Metcalfe-Smith et al. 2005). The periostracum varies from green, tan or brown with rays, while the nacre is white and

iridescent posteriorly (Metcalfe-Smith et al. 2005; COSEWIC 2013).The hinge teeth are strong and fully developed (COSEWIC 2013).

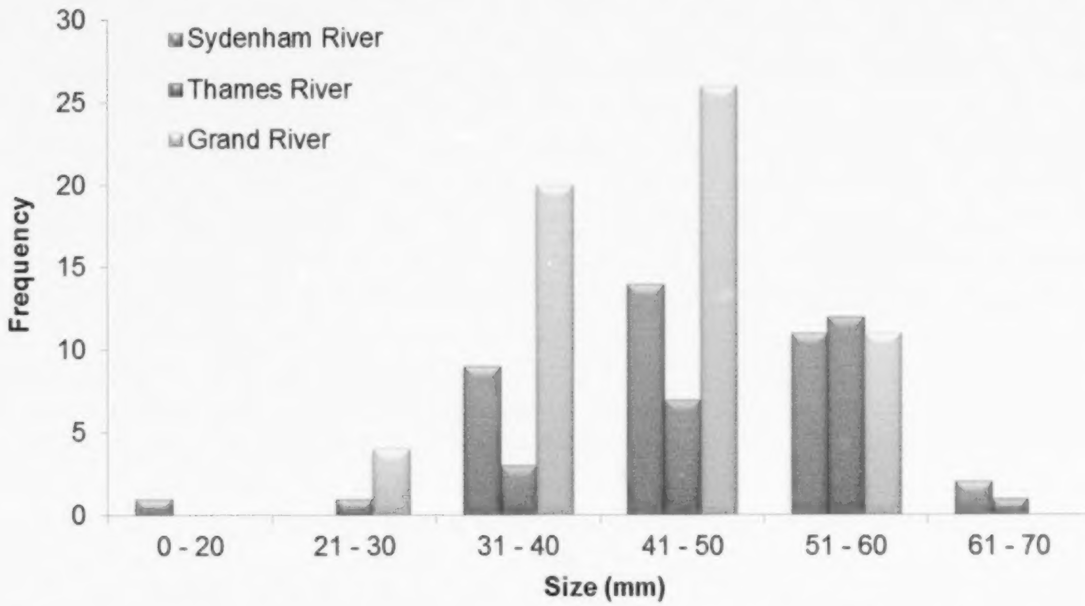

Figure 1. Size distribution of Threehorn Wartyback recorded from the Sydenham, Thames and Grand rivers [modified from COSEWIC (2013)].

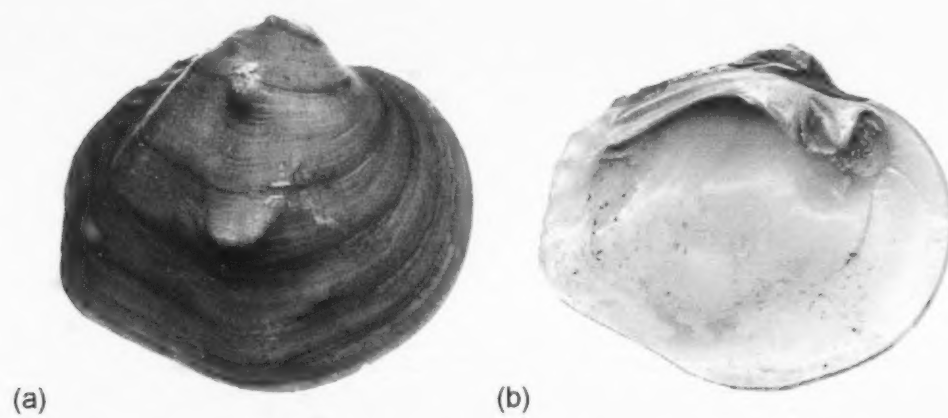

Figure 2. Threehorn Wartyback (a) exterior shell and (b) interior nacre. Photograph by Environment Canada, reproduced with permission.

Similar Species

Threehorn Wartyback is the only member of the genus *Obliquaria* known to occur in Canada (COSEWIC 2013). There are no morphologically similar species present in Canada, as Threehorn Wartyback can be easily distinguished by the presence of its characteristic alternating knobs.

Age and Growth

Threehorn Wartyback is considered to be a moderately short-lived species, with a maximum age estimate reported of 18 years (COSEWIC 2013). This age estimate is consistent for both Canadian (Morris, unpubl. data) and Ohio populations (Watters et al. 2009). Sixty valves sampled from the Grand River in 1997 were aged to determine the length at age relationship (DFO, unpubl. data; Figure 3). Mussel ages ranged from two (29 to 41 mm) to 14 years of age (54 mm). No additional information on age and growth patterns is available, locally or globally for this species.

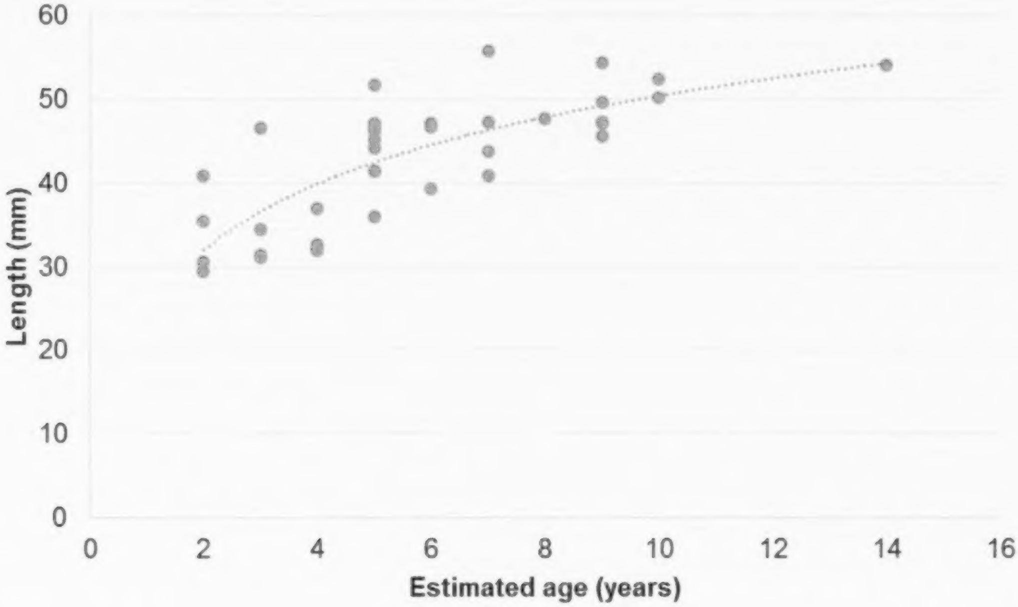

Figure 3. Length at age estimates for Threehorn Wartyback collected from the Grand River (DFO unpubl. data).

Diet

Like most other unionid mussels, Threehorn Wartyback is considered to be a filter feeder. Filter feeding (also called suspension feeding) is accomplished by using cilia to pump water through their incurrent siphon and over the gills. Particles are subsequently sorted by cilia on the gills and directed towards the mouth for consumption. In the early juvenile stage, when the mussel is most commonly buried in the substrate, food is obtained directly from the substrate in the form of algae and bacteria (Yeager et al. 1994). Species-specific dietary information is not available for Threehorn Wartyback.

Distribution

Globally, Threehorn Wartyback is considered secure (G5) and is distributed throughout much of the Mississippi River drainage (NatureServe 2014). It is considered to be possibly extirpated in Pennsylvania, critically imperilled in South Dakota, Iowa, and imperiled in Ohio and West Virginia (NatureServe 2014). In Canadian waters, it is currently limited to south-western Ontario, and specifically from the Sydenham, Thames and Grand rivers. Once thought to occupy Lake St. Clair, the Detroit River and the Canadian side of the western basin of Lake Erie, Threehorn

Wartyback is considered extirpated from these systems following the dreissenid mussel invasion (Gillis and Mackie 1994; Schloesser et al. 2006; COSEWIC 2013). Threehorn Wartyback is currently known from the American waters of western Lake Erie (D. Zanatta, unpubl. data).

CURRENT STATUS

Information within this report was drawn from data contained within the Lower Great Lakes Unionid Database as well as the COSEWIC status report (COSEWIC 2013), and unpublished data. For a detailed description of the Lower Great Lakes Unionid database and its historical data sources, see Metcalfe-Smith et al. (1998a). Ontario records generally resulted from formal studies directed at sampling unionids using both qualitative and quantitative methods. Sampling locations of all known sampling sites in Ontario (Figure 4) are shown to provide context of mussel sampling effort. Limited historical observations support the suggestion that Threehorn Wartyback has been historically rare in Canadian waters.

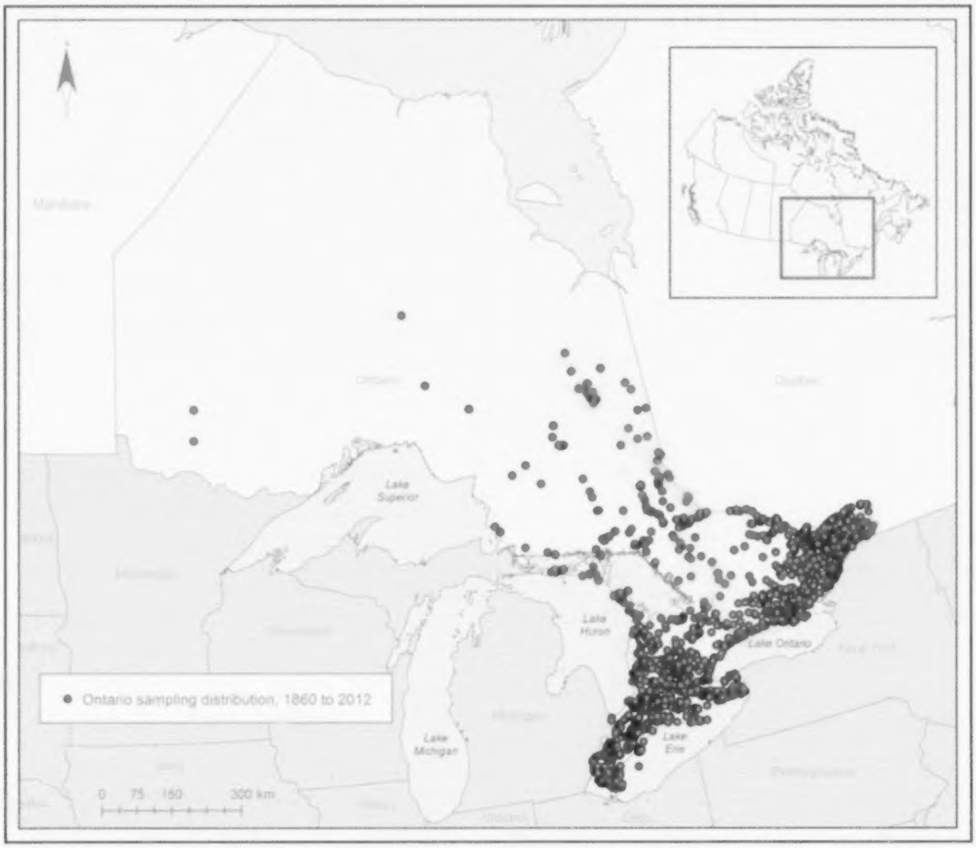

Figure 4. Distribution of all known historic and current freshwater mussel sampling in Ontario.

In Canada, the current and historic known distribution of Threehorn Wartyback is limited to five populations, one of which is currently considered to be extirpated and one that is represented by a single fresh shell. The Rondeau Bay location is represented by a single fresh shell recorded in 2011. Although Threehorn Wartyback is thought to be extirpated from the Canadian Great Lakes and connecting channels, 13 live individuals were recently detected in coastal wetlands and embayments in the western basin of Lake Erie and Sandusky Bay, Ohio (D. Zanatta,

unpubl. data). Extant populations include the Sydenham and Thames rivers (tributaries of Lake St. Clair) and the Grand River (tributary of Lake Erie; Figure 5). Live individuals have been recorded from all extant sites, with the greatest number of Threehorn Wartyback being recorded from the Sydenham River (n=73 since 1998). It should be noted that the following maps represent all current and historic records of Threehorn Wartyback, and may not accurately represent the current distribution. Substantial mussel sampling has occurred throughout Ontario; however, there has been limited sampling of the Great Lakes proper and connecting channels for mussels, as it is believed that most freshwater mussels are now extirpated from these areas following the dreissenid mussel invasion. Therefore, the following maps may be an underrepresentation of the current distribution, if Threehorn Wartyback is persisting, undetected from Canadian waters.

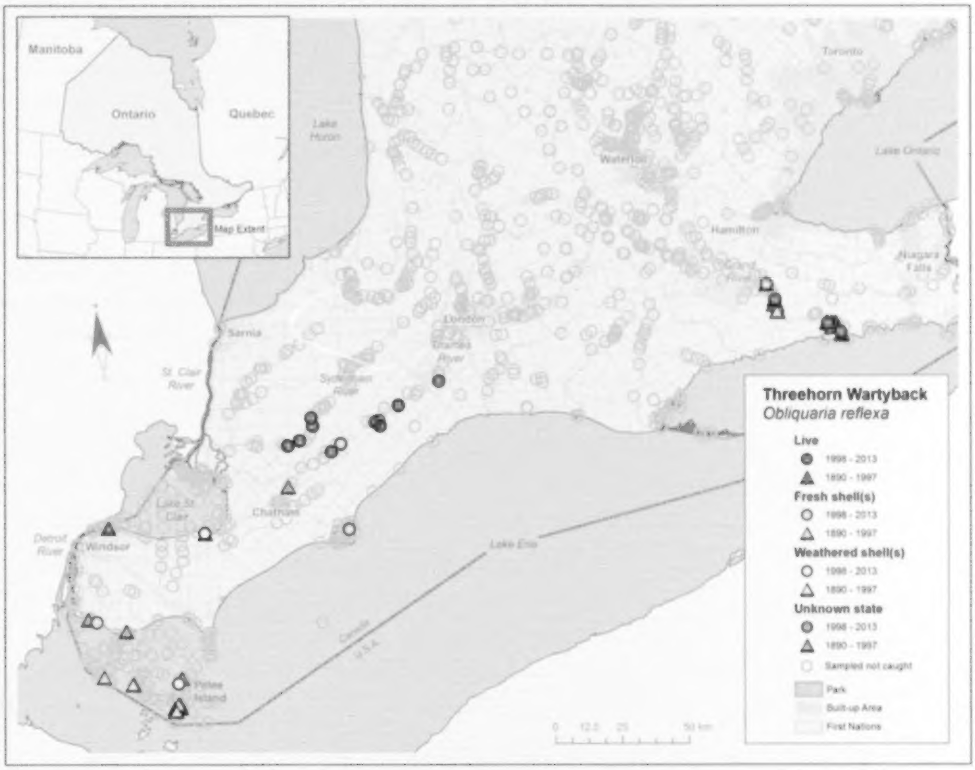

Figure 5. Distribution of Threehorn Wartyback in Canada.

Historically, Threehorn Wartyback were recorded from the Thames River (first record dated 1934), the Grand River (first record of a fresh shell from 1890) and the Great Lakes and their connecting channels (first record dated 1925). Historical records are generally comprised of museum records of valves or whole shells. Rarity of this species has yielded a mere 113 live individuals recorded in Canada since 1890, with the first confirmed live individual being recorded from the Detroit River in 1992.

A comparison between the maximal extent of occurrence (EO; 17 299 km^2) and the current EO (7032 km^2), using a minimum convex polygon approach, represents a 59% reduction in EO (COSEWIC 2013). In addition, the maximal and current index of occupancy (IAO) was estimated using a 2 km x 2 km grid cell approach (COSEWIC 2013). This comparison revealed a 73% reduction in current IAO when compared to maximal IAO (COSEWIC 2013).

Population Categorization

Characteristics to be considered when delineating populations include movement of the individual mussel (including movement of the host fishes), availability of suitable habitat between two locations, state of the Threehorn Wartyback recorded, and date of the record. In general, juvenile and adult mussels have very limited dispersal ability. Allen and Vaughn (2009) reported on both mean horizontal and mean vertical movement of adult Threehorn Wartyback. Threehorn Wartyback movement during five 11-day periods indicated that mean burrowing movement was approximately 1.4 cm (Allen and Vaughn 2009). Keeping in mind this limited ability for vertical movement, Threehorn Wartyback must rely on the dispersal abilities of their host fish to facilitate movement from one location to another. Therefore, for the purposes of this research document, populations have been delineated based on the ability of the host fish to move from one location where Threehorn Wartyback is known to occur to another. The putative host fishes for Threehorn Wartyback in Canada include Common Shiner (*Luxilus cornutus*) and Longnose Dace (*Rhinichthys cataractae*) (COSEWIC 2013). Refer to Host fishes section for additional information on host fish interactions and infestation experiments. Although there is currently no quantitative information in the literature on the movement ability of Common Shiner, a mark and recapture study by Hill and Grossman (1987) reported that Longnose Dace moved on average 13.4 m between captures (mean time interval between captures was 128 d).

In addition to host fish movement, we considered the state of the Threehorn Wartyback recorded (e.g., live individual, fresh shell, weathered shell), the date of the sampling event, and the amount of sampling effort that has occurred in a location when determining populations. Records consisting of live or fresh shells were considered valid when determining relevancy of records in population categorization. Weathered shells were not considered valid as a weathered shell can persist at a location for an undetermined amount of time, and would not necessarily provide evidence of a current population. Also, shells may move passively downstream, and generally older shells are likely to have moved greater distances making it difficult to determine the location the mussel occupied when living. Passive movement of shells would be relevant to any riverine population. The date of sampling event was also considered when determining the likelihood of a current population at a site. A record was considered current if it was recorded over the last ten years (since 2003).

Due to age and state of Canadian Threehorn Wartyback records from the Great Lakes and their connecting channels, we will not consider this location to represent a population. Although 13 live Threehorn Wartyback have been recently recorded from wetlands in American waters of the western basin of Lake Erie and Sandusky Bay, Ohio (D. Zanatta, unpubl. data), there are no Canadian records to indicate and extant population in Canadian waters. As there are no known historical records from Rondeau Bay and the only record from this area is a fresh shell collected in 2001, Rondeau Bay will not be considered a population. The following will be considered separate populations for the purposes of this research document: Sydenham River, Thames River, and Grand River.

Sydenham River

Threehorn Wartyback was first recorded from the Sydenham River in 1998 when one live individual was observed at Dawn Mills, and a second fresh shell was observed at Croton (Figure 6; Metcalfe-Smith et al. 2003). The Dawn Mills site has been re-sampled yearly from 2002 to 2009 and has resulted in the observance of 72 live individuals (33 recaptures and 39 new individuals; COSEWIC 2013). Threehorn Wartyback are known to occupy the reach of the Sydenham River between Dawn Mills and a site slightly upstream from Florence. Quantitative mussel surveys have been conducted in the Sydenham River; although population size estimates are not available for Threehorn Wartyback as only a single live individual was collected during these surveys. It is believed that recruitment is occurring in the Sydenham River population based on the current size frequency distribution (Figure 1), and the observation of a 15 mm individual (K. McNichols-O'Rourke, DFO, pers. obs.).

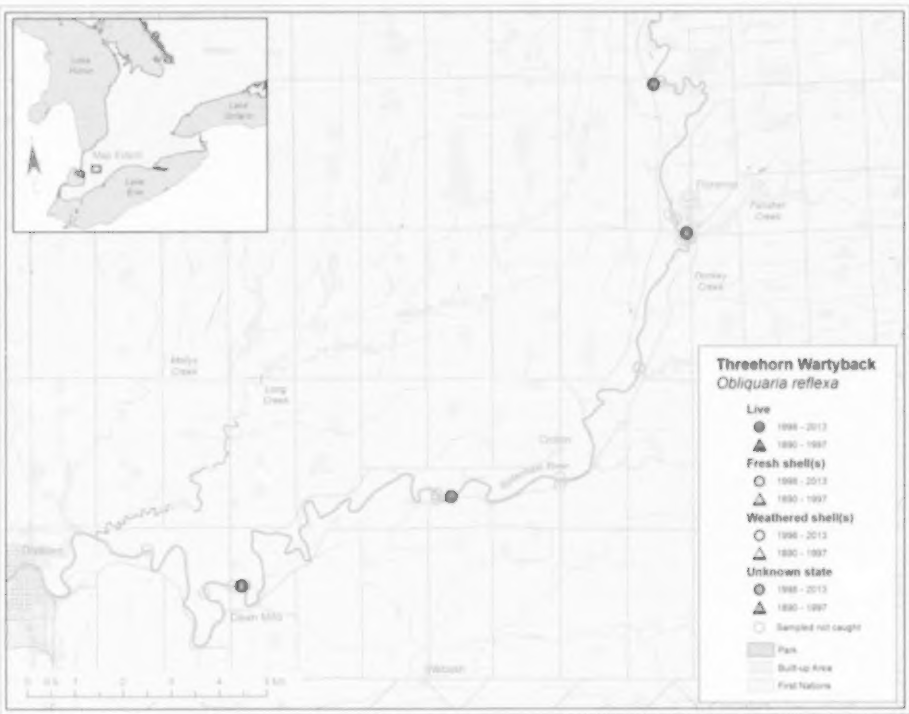

Figure 6. Distribution of all known current and historic Threehorn Wartyback records from the Sydenham River.

Thames River

There is a single historic record of Threehorn Wartyback in the Thames River, which was collected by J.P. Oughton in 1934. Threehorn Wartyback was not recorded from this system again until 1998 when one live individual and one fresh shell were recorded (Figure 7; Metcalfe-Smith et al. 1999). Five additional live Threehorn Wartyback were recorded from four sites in the Thames River in 2004 (John Schwindt, Upper Thames Conversation Authority, unpubl. data). DFO subsequently observed Threehorn Wartyback in the Thames River in 2005 (Morris and Edwards 2007) and 2010 (DFO, unpubl. data). Threehorn Wartyback are currently known to occupy a 100 km reach of the Thames River (COSEWIC 2013). A total of 30 live individuals have been collected from this system since 1998 (DFO, unpubl. data). Morris and Edwards (2007) estimated that the relative abundance of Threehorn Wartyback in the Thames River is 0.22% with an overall relative abundance of 0.024 animals/m^2 (COSEWIC 2013). Considering the known range of Threehorn Wartyback in this system, the population is estimated to be approximately 100 000 individuals (COSEWIC 2013).

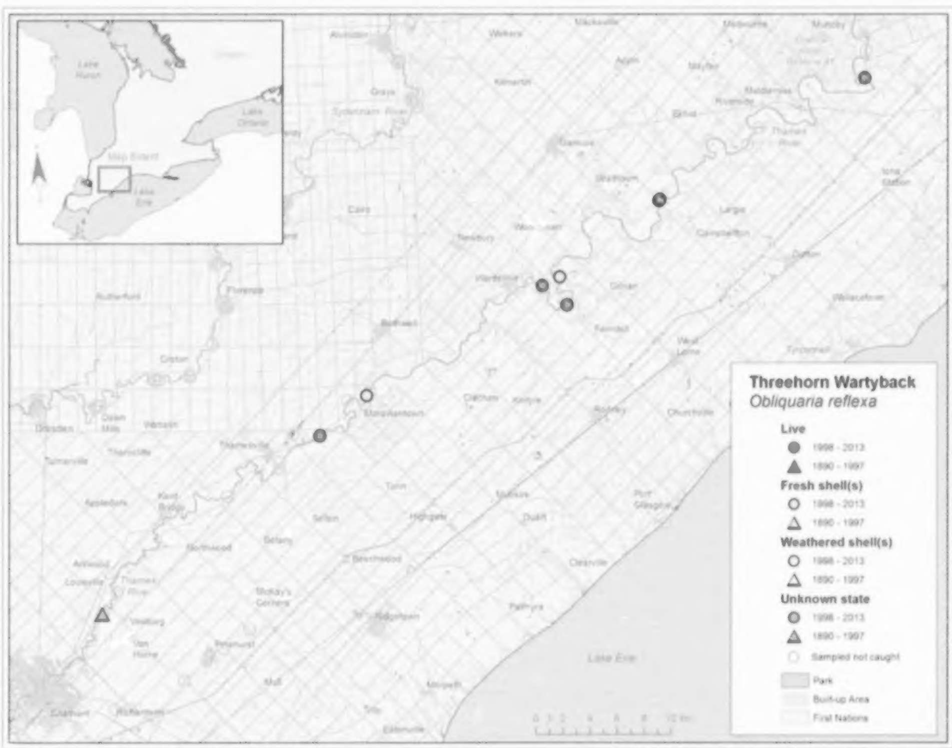

Figure 7. Distribution of all known current and historic Threehorn Wartyback records from the Thames River.

Grand River

Unlike the Sydenham and Thames rivers, historical records of fresh shells are available from museum collections for the Grand River. A total of 68 fresh shells were recorded from the Grand River between 1980 and 1988. The first live individuals were recorded in 1997 from Sulphur Creek, and the Grand River proper (Figure 8; Metcalfe-Smith et al. 2000). During a 1997 survey three live individuals, 40 fresh shells and 14 weathered shells were recorded from seven sites. One fresh shell was recorded in 2005, and one weathered shell was recorded in 2007. Most recently, four live individuals, five fresh shells and seven weathered shells were recorded from seven sites sampled in 2011 (McNichols-O'Rourke et al. 2012). Population size estimates are not available for Threehorn Wartyback in the Grand River.

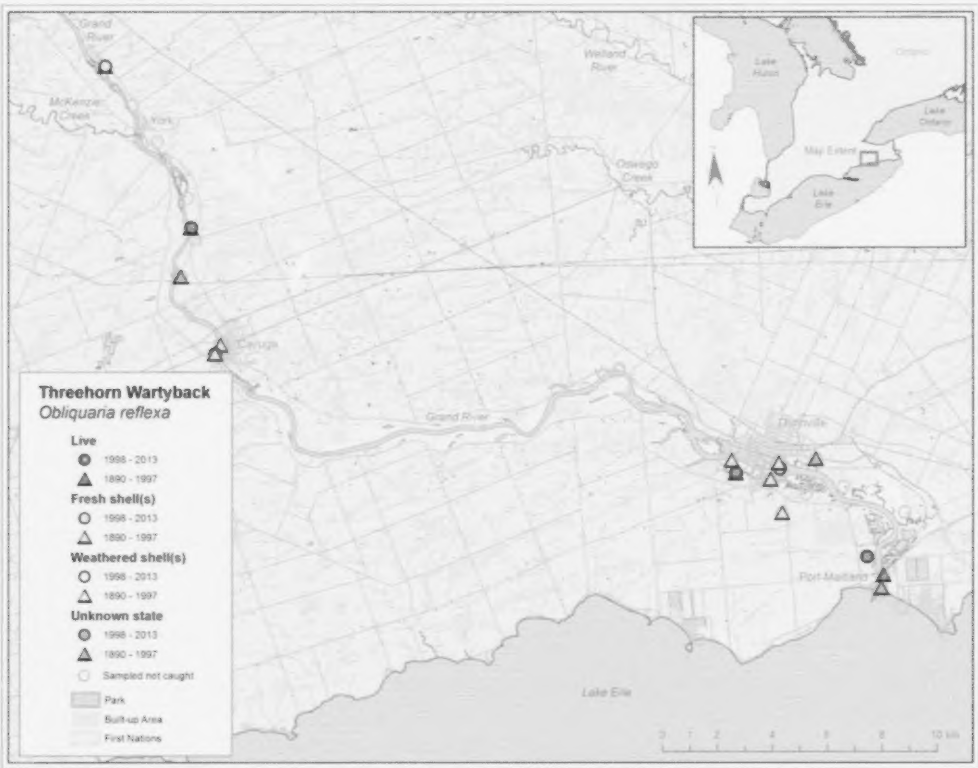

Figure 8. Distribution of all known current and historic Threehorn Wartyback records from the Grand River.

Rondeau Bay

One fresh Threehorn Wartyback shell was observed from Rondeau Bay in 2001 (collectors: D. Zanatta and D. Woolnough; Figure 9). This record represents the first, and only, record of Threehorn Wartyback in Rondeau Bay. Due to the scarcity of information related to Threehorn Wartyback in this system, Rondeau Bay will not be considered a population in the Population Status Assessment. Additional sampling in Rondeau Bay should be completed to determine whether a Threehorn Wartyback population persists in this system.

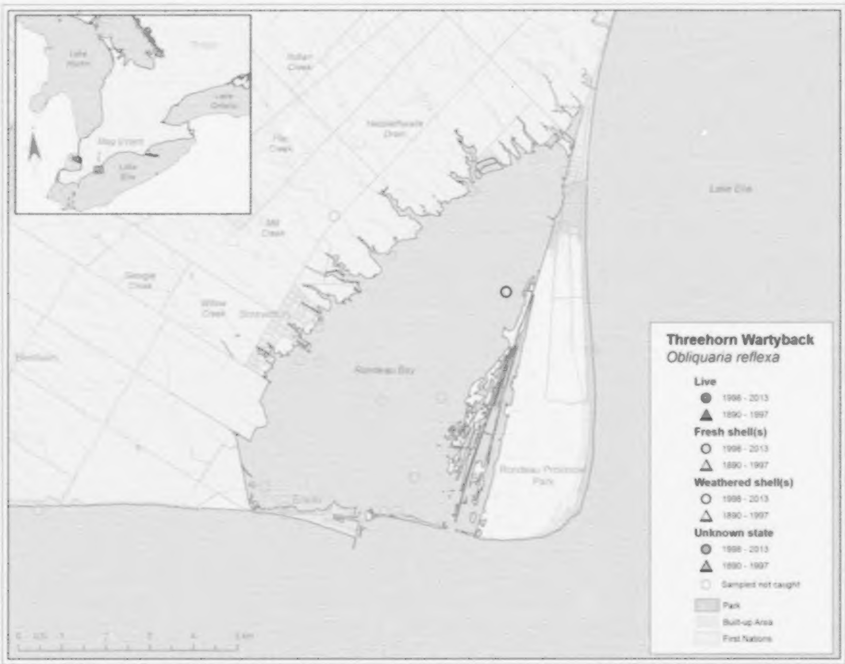

Figure 9. Distribution of all known current and historic Threehorn Wartyback records from Rondeau Bay.

Great Lakes and connecting channels

The first historic record of Threehorn Wartyback from the Great Lakes or their connecting channels was observed by M.E. Walker in 1925. The state or quantity of individuals observed is not available, but the location of this observation was near Oxley, on the northeastern shore of Lake Erie. Additional museum records provide evidence of shells recorded at various locations in Lake Erie including, Pelee Island (fresh shells collected from 1937 to 2005), East Sister Island (1967), Middle Sister Island (1952), The Meadows (2005) and the mouth of Big Creek (1982) (Figure 10). Despite these numerous shell collections, a live individual was not recorded until 1992 when Schloesser et al. (1998) recorded three live individuals from the Detroit River, near its confluence with Lake St. Clair. This record also represents the only live collection of Threehorn Wartyback from the Great Lakes and its connecting channels in Canadian waters. In 1998, additional sampling of previously visited sites yielded no observation of Threehorn Wartyback and it was concluded by investigators that unionids had been extirpated from the main river of the Detroit River as a result of the invasion of dreissenid (Schloesser et al. 2006). Due to a lack of current observations of live Threehorn Wartyback in Canadian waters of the Great Lakes and connecting channels, this population will not be included in the Population Status Assessment.

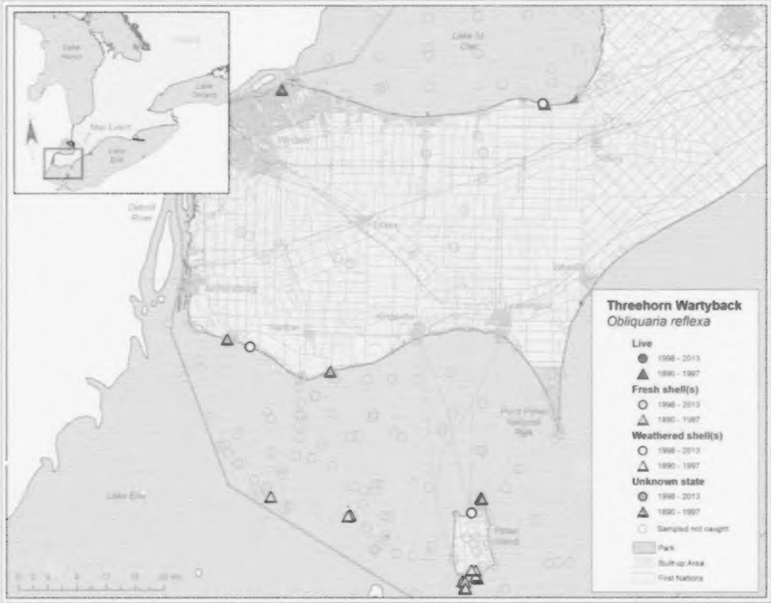

Figure 10. Distribution of all known current and historic Threehorn Wartyback records from the Canadian waters of the Great Lakes and connecting channels.

POPULATION STATUS ASSESSMENT

Assessment

To assess the Population Status of Threehorn Wartyback populations in Canada, each population was ranked in terms of its abundance and population trajectory (Table 1). Abundance was assigned as Extirpated, Low, Medium, High, or Unknown. Sampling parameters considered included sampling method, area sampled, sampling effort, and whether the study was targeting Threehorn Wartyback. The number of individual Threehorn Wartyback caught during each sampling period, as well as the state of the individual (live, fresh shell, or weathered shell) was then considered when assigning abundance. It is important to remember that abundance is based on Threehorn Wartyback records currently available.

The Population Trajectory was assessed as Decreasing, Stable, Increasing, or Unknown for each population based on the best available knowledge about the current trajectory of the population. The number of individuals caught over time for each population was considered. Trends over time were classified as Increasing (an increase in abundance over time), Decreasing (a decrease in abundance over time) and Stable (no change in abundance over time). If insufficient information was available to inform the Population Trajectory it was listed as Unknown.

Certainty has been associated with the Relative Abundance Index and Population Trajectory rankings and is listed as: 1=quantitative analysis; 2=CPUE or standardized sampling; 3=expert opinion.

Table 1. Relative Abundance Index and Population Trajectory of each Threehorn Wartyback population in Canada. Certainty has been associated with the Relative Abundance Index and Population Trajectory rankings and is listed as: 1=quantitative analysis; 2=CPUE or standardized sampling; 3=expert opinion.

Population	Relative Abundance Index	Certainty	Population Trajectory	Certainty
Sydenham River	Low	1 (timed search and quadrat surveys)	Unknown	3
Thames River	Low	1 (timed search and quadrat surveys)	Unknown	3
Grand River	Low	2 (timed search surveys and half-hectare plots)	Unknown	3

The Relative Abundance Index and Population Trajectory values were then combined in the Population Status matrix (Table 2) to determine the Population Status for each population. Population Status was subsequently ranked as Poor, Fair, Good, Unknown, or Not applicable (Table 3). Certainty assigned to each Population Status is reflective of the lowest level of certainty associated with either initial parameter (Relative Abundance Index, or Population Trajectory).

Table 2. The Population Status Matrix combines the Relative Abundance Index and Population Trajectory rankings to establish the Population Status for each Threehorn Wartyback population in Canada. The resulting Population Status has been categorized as Extirpated, Poor, Fair, Good, or Unknown.

		Population Trajectory			
		Increasing	**Stable**	**Decreasing**	**Unknown**
Relative Abundance Index	**Low**	Poor	Poor	Poor	Poor
	Medium	Fair	Fair	Poor	Poor
	High	Good	Good	Fair	Fair
	Unknown	Unknown	Unknown	Unknown	Unknown
	Extirpated	Extirpated	Extirpated	Extirpated	Extirpated

Table 3. Population Status of all Threehorn Wartyback populations in Canada, resulting from an analysis of both the Relative Abundance Index and Population Trajectory. Certainty assigned to each Population Status is reflective of the lowest level of certainty associated with either initial parameter (Relative Abundance Index, or Population Trajectory).

Population	Population Status	Certainty
Sydenham River	Poor	3
Thames River	Poor	3
Grand River	Poor	3

HABITAT REQUIREMENTS

Glochidium

To fully understand the habitat requirements of freshwater mussels, we must first understand their unique life cycle. Although Threehorn Wartyback are dioecious, they are believed to express very little sexual dimorphism (Watters et al. 2009). During the spawning period, males located upstream release sperm into the water column via the excurrent siphon. Females subsequently utilize their gills to filter the sperm from the water column, and the sperm is deposited in the posterior portion of the female gill in a specialized region where the ova are

fertilized. The fertilized ova are held until they reach a larval stage. Haag and Staton (2003) noted that Threehorn Wartyback only brood their young in the outer pair of gills, in what appear to be modified water tubes located approximately in the middle of each of the outer gills. It was also observed that the individual eggs of Threehorn Wartyback were bound tightly within the gills (Haag and Staton 2003). Threehorn Wartyback release their glochidia in a sausage-shaped conglutinate (Haag and Staton 2003). For Threehorn Wartyback, the mean number of conglutinates per individual has been recorded to be 8.1 conglutinates/individual (± 0.6), while the mean fecundity was observed from one site in Alabama as 25,767 (3250-82,500=range of observations) and one site in Mississippi as 40,975 (447 – 135,750=range of observations) (Haag and Staton 2003).

Freshwater mussels are often categorized in terms of their brooding and glochidial release patterns (Watters and O'Dee 2000). Two brooding strategies are long-term brooders (bradytictic) and short-term brooders (tachytictic). Threehorn Wartyback is classified as a short-term brooder, with glochidia being formed and released in May until the end of July (Clarke 1981; Watters et al. 2009; COSEWIC 2013). Collections of Threehorn Wartyback in May in June in Mississippi resulted in a high percentage of gravid females (97%; Haag and Staton 2003). Gravid females have also been observed in the Sydenham River in June when water temperatures were approximately 20°C (COSEWIC 2013).

Regardless of brooding strategy, once females release their glochidia they must encyst on the gills of an appropriate host fish (Kat 1984). Although it has been suggested that Threehorn Wartyback may not require a host fish to complete its life cycle (Utterback 1916), there has been no additional support for this suggestion in the literature. Glochidial mortality is currently unknown but it is estimated that as little as 0.001% of glochidia successfully attach to an appropriate host fish (Bauer 2001). Metamorphosis from glochidia to juvenile cannot occur without a period of encystement, which has been recorded to last 17-19 days post attachment (COSEWIC 2013).

Host fishes

Infestation experiments to determine host fish for Threehorn Wartyback in Canada have not occurred, but Common Shiner (*Luxilus cornutus*), Longnose Dace (*Rhinichthys cataractae*) and Silverjaw Minnow (*Notropis buccata*) have been identified to be appropriate host fish in the United States (see Watters et al. 2009 for species-specific references). In addition, Barnhart and Baird (2000) recorded a natural infestation of Threehorn Wartyback on Goldeye (*Hiodonta alosoides*). They concluded that this host association was highly probable as the glochidia were numerous and had grown while encysted (Barnhart and Baird 2000).

Complete distributional overlap with the extant range of Threehorn Wartyback in Canada does exist for Common Shiner, while partial distributional overlap exists for Longnose Dace (Mandrak and Crossman 1992 ; Scott and Crossman 1973; Holm et al. 2010; DFO, unpubl. data). There is no distributional overlap between Threehorn Wartyback and Goldeye and Silverjaw Minnow in Canada. Goldeye's currently known distribution is restricted to warm, pelagic waters of large streams and turbid lakes of northern Ontario (Holm et al. 2010), while Silverjaw Minnow is not currently known from Canadian waters. Neither species is a candidate functional host fish for Threehorn Wartyback in Canada. However, the Canadian distribution of Mooneye (*Hiodon tergisus*) (Holm et al. 2010), a species closely related to Goldeye does partially overlap Threehorn Wartyback distribution. Although there are no known studies on Mooneye as the functional host for Threehorn Wartyback, the potential relationship should be investigated. Common Shiner have been described as inhabiting cool waters of shallow pools and run in streams, while Longnose Dace inhabit fast-flowing water of streams (Holm et al. 2010). The overlap in distribution provides circumstantial evidence to the probable host-mussel relationship between Threehorn Wartyback and Common Shiner, and Longnose Dace.

Many factors must be considered when discussing the suitability and probability of a successful host fish encounter. The host fish must not only be present in the system in sufficient numbers, but must be of appropriate age, health and immunity to be susceptible to infestation and act as a candidate host fish. Specific criteria related to these factors are currently unknown for Threehorn Wartyback and these two probable host fishes should be the focus of future studies.

Many species of freshwater mussels have evolved complex host attraction strategies to increase the probability of encountering a suitable host (Zanatta and Murphy 2006). Threehorn Wartyback does not appear to utilize an active host-attraction strategy, and does not appear to have a lure to attract their host (Zanatta and Murphy 2006). However, it has been noted that Threehorn Wartyback releases club-shaped conglutinates (Barnhart and Baird 2000; Watters et al. 2009). A predatory response from the host fish causes the host to ingest the conglutinate, resulting in the release of, and subsequent attachment of, the glochidia.

Regardless of the method of exposure and attachment, glochidia will remain encysted on the host fish until they metamorphose into juveniles. The proportion of glochidia that survive to the juvenile stage is estimated to be as low as 0.000001% (Jansen and Hanson 1991; COSEWIC 2006b, 2007). A survival tactic to overcome this increased level of mortality is to produce very high numbers of glochidia. Encystement is an obligate step in the life cycle of Threehorn Wartyback and development will not occur in the absence of this phase. The gills of the appropriate host fish can be considered a habitat requirement for the glochidial life stage of Threehorn Wartyback.

Juvenile

Subsequent to metamorphoses, juvenile freshwater mussels are released from the gills of the host fish and burrow into the substrate until maturity. Time to maturity can vary from one mussel species to another and accurate estimates are not known for most species. It is difficult to classify required habitat for juvenile mussels because they are difficult to detect, as they have a tendency to burrow (Schwalb and Pusch 2007). Once sexually mature they emerge from the substrate to participate in gamete exchange (Watters et al. 2001). Threehorn Wartyback age at maturity is currently unknown.

Adult

General characteristics

Threehorn Wartyback is most commonly found in large rivers with stable gravel, sand and mud substrates with moderate current; however, it may also be found in shallow embayments and reservoirs with almost no current (Metcalfe-Smith et al. 2005). It has been suggested that Threehorn Wartyback can tolerate a wide range of water temperatures, depths, substrates and flows (COSEWIC 2013).

Temperature

A study focusing on the physiological response of mussels to various water temperatures, determined that Threehorn Wartyback showed no change in body condition at 5, 15, 25 and 35°C (Spooner et al. 2005). However, Threehorn Wartyback did show elevated glycogen levels (a surrogate for long-term physiological condition), at 35°C, indicating that temperatures over 35°C may have long-term effects on Threehorn Wartyback (Spooner et al. 2005). It was suggested that additional temperature regimes should be tested to determine temperature tolerances for Threehorn Wartyback; although temperature does not appear to be a limiting factor for this species (COSEWIC 2013). Live Threehorn Wartyback recorded in Ontario from 1997 to 2013 were recorded from sites where the water temperature ranged from 18 to 27°C.

However, water temperatures have only been recorded at time of capture and would not reflect the wide range of water temperatures experienced by the mussel throughout the seasons.

Depth

Although Threehorn Wartyback has been found in water as deep as 6-7 m in other parts of its range (Parmalee and Bogan 1998; Georgia Museum of Natural History 2013), limited deep waters sampling has occurred in Ontario waters to confirm usage of deep water habitats by Threehorn Wartyback. A scuba diving survey of the lower Grand River in 1997 did record one live Threehorn Wartyback in water 4 m deep, and another in 5 m (Metcalfe-Smith et al. 1998b). Sites where live Threehorn Wartyback have been recorded in Ontario had an average site water depth of 0.64 ± 0.22 m (DFO, unpubl. data).

Substrate

Threehorn Wartyback have been described as occupying areas with stable gravel, sand or mud substrates (Metcalfe-Smith et al. 2005) and areas with muddy sand, or cobble (Watters et al. 2009). Limited habitat information is available from the sites where live Threehorn Wartyback have been recorded between 1997 and 2013 from the Grand, Sydenham and Thames rivers (Figure 11). The percent composition of various substrate types were estimated during site visits and it was found that majority of sites were composed of a combination of boulder, rubble, gravel and sand (Figure 11). Very few sites were described as being composed of silt, clay, much or detritus.

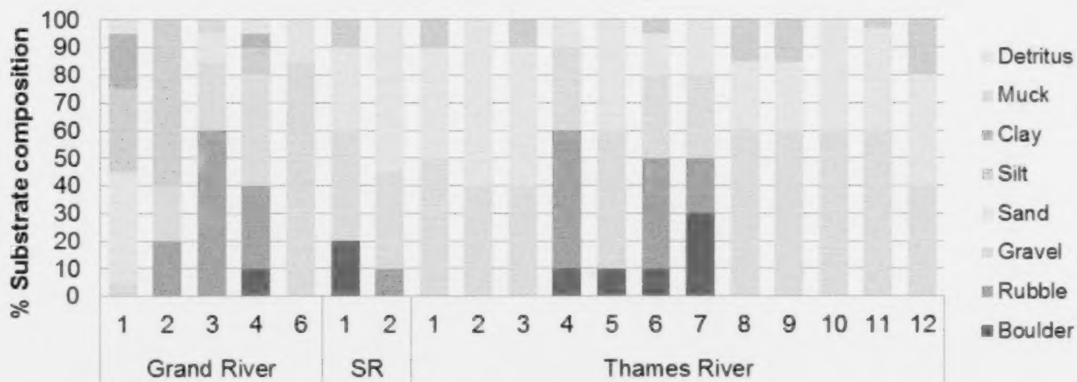

Figure 11. Substrate composition (%) recorded at sites where live Threehorn Wartyback were recorded from 1998-2013 in the Grand River, Sydenham River (SR), and Thames River.

Flow

There is a paucity of site specific flow information from locations where live Threehorn Wartyback have been recorded in Ontario. Live Threehorn Wartyback (n=3) were recorded from a single site on the Thames River in 2010, at which the average site water velocity was recorded as 0.56 ± 0.04 $m \cdot s^{-1}$ (DFO, unpubl. data). Three sites on the Sydenham River where live Threehorn Wartyback were recorded measured water velocity to be 0.237 ± 0.043 $m \cdot s^{-1}$ (Metcalfe-Smith et al. 2007). Subsequently, one live Threehorn Wartyback was recorded from the Sydenham River in 2013 and the water velocity was recorded as 0.37 $m \cdot s^{-1}$ (DFO, unpubl. data). As noted above, water flows were recorded at time of capture and are more likely indicative of low flow as sampling generally occurs when flows are at a minimum. This range would not represent the range of flows the mussel would experience throughout the year.

Functions, Features and Attributes

A description of the functions, features, and attributes associated with Threehorn Wartyback habitat can be found in Table 4. The habitat required for each life stage has been assigned a function that corresponds to a biological requirement of Threehorn Wartyback. In addition to the habitat function, a feature has been assigned to each life stage. A feature is considered to be the structural component of the habitat necessary for the survival or recovery of the species. Habitat attributes have also been provided, which describe how the features support the function for each life stage. Optimal habitat attributes from the literature for each life stage have been combined with habitat attributes from current Canadian records (recorded from 1997 to present) to show the maximum range in habitat attributes within which Threehorn Wartyback may be found (see Table 4 and references therein). This information is provided to guide any future identification of critical habitat for this species. It should be noted that habitat attributes associated with current records may differ from those presented in the scientific literature as Threehorn Wartyback may be currently occupying areas where optimal habitat is no longer available.

Residence

Residence is defined in SARA as "dwelling-place, such as a den, nest or other similar area or place, that is occupied or habitually occupied by one or more individuals during all or part of their life cycles, including breeding, rearing, staging, wintering, feeding or hibernating". Residence is interpreted by DFO as being constructed by the organism (DFO 2010). In the context of the above narrative description of habitat requirements during glochidial, juvenile and adult life stages, Threehorn Wartyback does not construct a residence during its life cycle.

POPULATION SENSITIVITY TO PERTURBATION

There was insufficient information on the life history of Threehorn Wartyback in Canada to complete a population model of the species. For use in such data-poor scenarios, Young and Koops (2011) used a population matrix model framework to explore the sensitivity of Unionid mussel populations to perturbations.

Sensitivity was quantified using elasticities, which can be used to describe the expected percent change in the long-term population growth rate as a result of a percent change in a vital rate (Caswell 2001). A range of possible Unionid life histories were classified into groups with similar elasticities. It was found that sensitivity groups could be predicted if certain vital rates were known to be on either the high or the low end of the parameter range. Life histories were classified into the following groups:

- Reproduction dominant: population growth was most sensitive to perturbation or uncertainty in age at maturity; glochidial survival and fecundity were more influential in this group than in others.
- Adult survival dominant: adult survival influenced population growth much more than juvenile survival. Remaining vital rates were relatively less important.
- Juvenile survival dominant: population growth was most influenced by juvenile survival.

Table 4. Summary of the essential functions, features and attributes for each life stage of Threehorn Wartyback. Habitat attributes from published literature, and habitat attributes recorded during recent Threehorn Wartyback surveys (recorded since 1997) have been combined to derive the habitat attributes required for the delineation of critical habitat (see text for a detailed description of categories).

			Habitat Attributes		
Life Stage	**Function**	**Feature(s)**	**Scientific Literature**	**Current Records**	**For Identification of Critical Habitat**
Spawning and fertilization (short-term brooder: glochidia being formed and released in May until the end of July)	Reproduction	Large rivers with moderate flow		• Gravid female Threehorn Wartyback have been observed in the Sydenham River in June at temperatures of ~20°C (Castanon, pers. comm. 2011 in COSEWIC 2013)	• Same habitat as adult
Encysted glochidial stage on host fish until drop off	Development	Appropriate host fish(es)	• Infestation experiments have determined that Common Shiner, Longnose Dace, and Silverjaw Minnow are appropriate host fishes in United States (see Watters et al. 2009 for species-specific references) • There is a single record of a natural infestation of Threehorn Wartyback on the gills of Goldeye in Missouri, USA (Barnhart and Baird 2000)	• There are no records of natural or laboratory infestations of Threehorn Wartyback glochidia on gills of putative host fishes	• Presence of sufficient host fish (putative host fishes in Canadian waters are Common Shiner and Longnose Dace)
Adult/juvenile	Feeding Cover Nursery	Large rivers with moderate flow	**General** • Categorized as occupying large rivers with moderate current, and shallow embayments and reservoirs with almost no current (Metcalfe-Smith et al. 2005; Watters et al. 2009)	• General characteristics taken from the literature supported by recent reports of live individuals	
			Substrate • Threehorn Wartyback occupies areas with stable gravel, sand or mud substrates (Metcalfe-Smith et al. 2005) and areas with muddy sand, or cobble (Watters et al. 2009)	• The majority of sites where live Threehorn Wartyback were recorded were composed of a combination of boulder, rubble, gravel and sand (DFO, unpubl. data)	• Most often found in areas where the substrate is composed of boulder, rubble, gravel and sand, or a combination thereof

Life Stage	Function	Feature(s)	Habitat Attributes: Scientific Literature	Habitat Attributes: Current Records	Habitat Attributes: For Identification of Critical Habitat
			Depth • Threehorn Wartyback has been found in water as deep as 6-7 m in other parts of its range (Parmalee and Bogan 1998; Georgia Museum of Natural History 2013)	• A scuba diving survey of the lower Grand River in 1997 recorded one live Threehorn Wartyback in water 4 m deep, and another in 5 m (Metcalfe-Smith et al. 1998b). • Sites where live Threehorn Wartyback have been recorded in Ontario had an average site water depth of 0.64 ± 0.22 m (DFO, unpubl. data).	
			Presence of dreissenid mussels • Introduction and establishment of dreissenid mussels has negatively affected freshwater mussels in the Great Lakes	• Zebra Mussel (*Dreissena polymorpha*) present at Thames River site (TR-50) in 2010 where live Threehorn Wartyback were recorded (DFO, unpubl. data) • Zebra Mussel present from the Fanshawe Reservoir in London, downstream to near Thamesville, and are likely present all the way to the river mouth (Morris and Edwards 2007) • Zebra Mussel present in the Grand River up to the Dunnville Dam (G. Mackie, pers. comm.)	

The maximum observed lifespan in Ontario is 18 years (COSEWIC 2013), which classifies it as a "short lived" species (values used in previous modelling were 10 years and 50 years for "low" and "high" values, respectively). It therefore must also have "early" age at maturity due to how maturity was defined in previous modelling. Mean fecundity of Threehorn Wartyback was found to be 40,975 in Massachusetts and 25,767 in Alabama, and did not exceed 136,000 in either state (Haag and Staton 2003). We therefore classify Threehorn Wartyback as having "low" fecundity. Using the classification system from Young and Koops (2011), Threehorn Wartyback falls into the adult survival dominant group. An updated version of this classification system (DFO unpubl. data) also suggests that the species may fall into a fourth "low sensitivity" group. This group is similar to the adult survival dominant group but with lower sensitivity to adult survival (i.e., population growth is less sensitive to all vital rates compared to other groups). In this group, population growth is equally sensitive to changes in adult survival, juvenile survival, and lifespan.

Note that sensitivity analyses are meant to compare expected responses in population growth to changes in vital rate. Pertinent threats to the species may affect life stages not identified as being most sensitive to perturbation.

THREATS

In the past 30 years, species diversity and abundance of native freshwater mussels has declined throughout Canada and the United States (Williams et al. 1993). The greatest limiting factors to the stabilization and growth of freshwater mussel populations in Canada are largely attributed to decreases in the quantity and quality of available freshwater mussel habitat and the introduction and establishment of dreissenid mussels. The historic vast distribution of freshwater mussels in the Great Lakes and its connecting channels has been devastated by the introduction of dreissenid mussels, and many of the areas once inhabited by freshwater mussels no longer provide suitable habitat. In addition, evidence suggests that decreases in water quality, specifically increased turbidity and suspended solids, increased nutrient loading, and increased levels of contaminants and toxic substance are also limiting the distribution of freshwater mussels. These declines in water quality are the result of activities such as dam construction and impoundments, channel modifications (e.g., channelization, dredging, snagging) and land-use practices (e.g., farming, mining, construction) (Bogan 1993; Williams et al. 1993; Watters 2000). Impoundments typically result in siltation, pollutant accumulation and nutrient-poor water, while dams alter flow and temperature regimes and separate mussels from their host fish (Bogan 1993; Watters 2000). Land-use practices such as farming, and construction usually result in the runoff of sediments, pollutants and salt into streams (Bogan 1993; Watters 2000). Urbanization and both residential and commercial development may be negatively affecting Threehorn Wartyback across much of its known distribution, contributing to further decreases in water quality by increasing sedimentation from land development, increasing nutrient and contaminant inputs and modifying natural systems (e.g., creation of dams).

A wide variety of threats negatively affect Threehorn Wartyback across its range. Our knowledge of threat impacts on Threehorn Wartyback populations is limited to general documentation, as there is a paucity of threat-specific cause and effect information in the literature. It is important to note the threats discussed below may not always act independently on Threehorn Wartyback populations; rather, one threat may directly affect another, or the interaction between two threats may introduce an interaction effect. It is quite difficult to quantify these interactions; therefore, each threat is discussed independently.

Contaminants and toxic substances

Freshwater mussel life history characteristics make them particularly sensitive to increased levels of sediment contamination and water pollution. Adult mussels feed primarily by filter feeding, while juveniles remain burrowed deep in the sediment feeding on particles found within the sediment. As mussels generally display little movement, they tend to accumulate deleterious substances from their environment, one of the reasons they are prime candidates for studies in environmental ecotoxicology. Toxic chemicals from both point and non-point sources are believed to be one of the major threats to mussel populations today (Strayer and Fetterman 1999). In rural areas contaminants and toxic substances can originate from agricultural practices, while inputs in urbanized areas generally include sewage pollution from outflows or stormwater runoff, and toxic pollutants that enter the sewer system from industrial operations. Of increasing concern is the application of road salts as a de-icing or anti-icing chemical.

A study conducted by Environmental Canada in 2001 analyzed the sediment quality in the mouths of all tributaries to Lake St. Clair and Lake Erie (Table 5; Dove et al. 2002). From the results of this study, we can see that many of the tributaries within the known distribution of live Threehorn Wartyback records showed exceedances of both federal and provincial standards for various metals and pesticides. However, all samples from all three sites did not exceed federal and provincial standards for PAHs or PCBs (Dove et al. 2002).

Table 5. Summary of sediment contamination at the mouths of various tributaries of Lake St. Clair and Lake Erie where live Threehorn Wartyback have been recorded. Blank cells represent samples where there was no detection. Table reproduced from Dove et al. (2002; TEL=federal threshold effect level, LEL=provincial lowest effect level).

	Exceedance of standards*			
River	**Total PAHs**	**Total PCBs**	**Metals**	**Pesticides**
Grand River			Copper, Manganese: LEL Arsenic, Lead, Zinc: TEL and LEL	Total DDE: TEL
Sydenham River			Iron, Manganese, Nickel: LEL Arsenic, Chromium , Copper, Zinc: TEL and LEL	
Thames River			Copper, Iron, Manganese: LEL Arsenic, Lead: TEL and LEL	Total DDE: TEL

*Federal levels (TEL) set by Environment Canada; Provincial levels (LEL) set by the Ministry of the Environment

The effects of heavy metals on mussels have been reviewed by Fuller (1974) and it was concluded that substances such as arsenic, cadmium, chlorine, copper, mercury and zinc can be toxic to freshwater mussels because they accumulate these substances from their environment. This toxicity may be increasingly relevant to glochidia and juveniles. Interestingly, a study on the sensitivity of freshwater mussels to copper indicated that conglutinates were found to provide significant protection from acute copper exposure when compared to freed glochidia (Gillis et al. 2008); therefore, this reproductive strategy, utilized by Threehorn Wartyback, may afford the glochidia some protection from contaminants. There is also an ever-

growing body of literature indicating that freshwater mussels are sensitive to ammonia (Augspurger et al. 2003; Bartsch et al. 2003; Mummert et al. 2003).

The application of road salts as a de-icing or anti-icing chemical has been highlighted as an increasing area of concern for our lakes and streams (Environment Canada 2001). Road salts enter the surface water and groundwater after snow melt and can lead to the salinization of our lakes, rivers, and streams (Demers and Sage Jr. 1990). A study was recently completed assessing the long-term trend in chloride concentrations in areas known to be inhabited by mussel species at risk in southwestern Ontario, indicating that a significant increase in chloride concentration was observed at 96% of the 24 long-term (1975-2009) monitoring sites (Todd and Kalteneckerm 2012). An additional study completed by Gillis (2011) determining the level of acute toxicity of NaCl for glochidia of various species of mussel (including two species endangered in Canada), reported that chloride data collected from mussel habitats reached levels of acute toxicity for glochidia.

Effluents from municipal treatment plants are known as a major source of pollution, releasing metals, PAHs, pharmaceuticals and various endocrine-disrupting compounds into waterways (Chambers et al. 1997). In addition, numerous studies have reported the negative effects of municipal effluents on freshwater mussel health (Gagné and Blaise 2003; Gagné et al. 2004; Gagné et al. 2011; Gillis 2012). Gagné and Blaise (2003) reported that freshwater mussels caged downstream from a municipal water treatment facility with primary treatment were exposed to estrogenic chemicals present in the municipal effluent, which may alter the normal metabolism of serotonin and dopamine, two chemicals involved in sexual differentiation. This is further supported by Gagné et al. (2011) who presented evidence that exposure to municipal effluent may lead to feminization of wild freshwater mussels and be disrupting gonadal physiology (Gagné et al. 2011). In the Grand River, Flutedshell (*Lasmigona costata*) downstream of 11 municipal wastewater treatment plants were shown to have reduced condition factor, and did not live as long (significantly reduced mean age), as well as exhibited negative immune status when compared to mussels upstream of the outfall (Gillis 2012) .

Nutrient loading

Agriculture, the primary land use in many southwestern Ontario watersheds, appears to be contributing to poor water quality through agricultural runoff and manure seepage (Grand River Conservation Authority 1997; Thames River Recovery Team 2005; MacDougall and Ryan 2012). Particularly relevant to freshwater mussels are the indirect effects of increased nutrient loading, such that, increases in nutrient levels can lead to increased algal growth. Once algal masses senesce, the oxygen supply in the water column is used for the decomposition process, leading to decreased levels of available oxygen. Strayer and Fetterman (1999) identified increased nutrient loads from non-point sources, and especially from agricultural activities as a primary threat to freshwater mussels.

Tile drainage, wastewater drains, manure storage and spreading may contribute to poor water quality in watersheds dominated by agricultural lands. Increased application of nutrients (nitrogen and phosphorus) as fertilizer and manure was the main driver for the declining trend in the performance index for water quality throughout Canada (Environmental sustainability of Canadian Agriculture: Agri-environmental indicator report series – Report No. 3; Accessed: 11 November 2013). Specifically, the Thames, Sydenham, and Grand rivers face increased pressure from agricultural activities, and often show high nutrient levels with total phosphorus levels often exceeding the provincial water quality objective (PWQO) of 30 µg/L (St. Clair Region Conservation Authority 2009; MacDougall and Ryan 2012). Water quality monitoring in Sydenham River reported total phosphorus concentrations from 30 µg/L to 200 µg/L (St. Clair Region Conservation Authority 2009). Concentrations of total phosphorous, associated with agricultural runoff, continue to increase in the east branch of the Sydenham River, and may be

affecting Threehorn Wartyback. An intensive water quality monitoring study focusing on the lower Grand River was conducted in 2003 and 2004 (MacDougall and Ryan 2012). This study indicated that of the 402 water samples collected throughout the lower Grand River that only six of these samples fell below the PWQO for total phosphorus (MacDougall and Ryan 2012). In addition, high nutrient levels in the Grand River cause anoxic conditions during summer months within the impounded region upstream of the Dunnville Dam (Grand River).

Turbidity and sediment loading

Increases in turbidity, and the subsequent decrease in silt-free habitats has reduced the quantity and quality of freshwater habitat across southwestern Ontario. Increased siltation affects freshwater mussels by clogging siphons, hindering the intake of oxygen and impeding reproductive functions (Strayer and Fetterman 1999). Increased suspended solids in the water column can clog the gill structures and ultimately suffocate the mussel. Furthermore, the reproductive cycle of Threehorn Wartyback requires a visual predator to prey on, and ingest the conglutinate to become infested with glochidia. Extreme levels of siltation would decrease the likelihood that the host fish will be able to locate the conglutinate.

Increased sediment loading is often associated with increased agricultural land use. Increased agricultural land use can also lead to riparian vegetation clearing or unrestricted livestock access to the river leading to poor water quality with increased sediment loads (Water Quality Branch 1989a). Agricultural practices and increased tile drainage results in large inputs of sediments to the watercourse. On a much smaller scale, in-water projects without sedimentation controls may cause temporary turbidity increases in the waterway.

Portions of the Thames and Sydenham rivers flow through areas of prime agricultural land in southwestern Ontario. It is estimated that over 85% of the land in the Sydenham River and 88% of the land in the lower Thames River is used for agricultural purposes and large extents of these rivers have little to no riparian vegetation (Dextrase et al. 2003; Taylor et al. 2004). Dextrase et al. (2003) reported suspended solid levels in the Sydenham River to be as high as 900 $mg \cdot L^{-1}$, which would undoubtedly negatively affect the freshwater mussel assemblage.

Another watershed greatly affected by increased turbidity is the Grand River. It is believed that poor water quality and increased sediment loads in this watershed have resulted from riparian vegetation clearing and increased livestock access to the river (Water Quality Branch 1989a). In addition, impoundments present in the lower Grand River, poor land use and re-suspension of sediment by the feeding behavior of Common Carp (*Cyprinus carpio*) contribute to increased levels of turbidity. The effects of increased agricultural land use in the Grand River on Threehorn Wartyback are not known.

Although, it is known that increases in turbidity and decreases in silt-free habitats reduce water quality for freshwater mussels, it is currently unknown what effects these environmental changes may have on Threehorn Wartyback. It has been suggested that sedimentation effects (i.e. the accumulation of silt on the streambed that may reduce flow rates and dissolved oxygen concentrations below the surface) may have a greater impact on species that tend to borrow completely in the substrate, such as Threehorn Wartyback (Österling et al. 2010; COSEWIC 2013). Research is required to determine turbidity and sedimentation tolerance levels of Threehorn Wartyback. However, increased turbidity will undoubtedly affect the host fish's ability to locate the mussel, and would decrease the probability of a host fish encounter and glochidial transfer.

Invasive species

Dreissenid mussels, Zebra Mussel and Quagga Mussel (*Dreissena bugensis*), have severely affected native, lacustrine freshwater mussel populations. The invasion and spread of these

invasive species throughout the Great Lakes and their tributaries has decimated many native freshwater mussel populations (Schloesser and Nalepa 1994; Nalepa et al. 1996; Ricciardi et al. 1996; Schloesser et al. 1996; Schloesser et al. 1998; Zanatta et al. 2002). Zebra Mussel compete with native mussel species for space and food and can attach to freshwater mussel shells, impairing movement, burrowing, feeding, respiration, reproduction and other physiological activities (Mackie 1991; Haag et al. 1993; Baker and Hornbach 1997). This typically results in the death of the unionid mussel. Zebra Mussel exhibit rapid population growth and are able to eliminate entire unionid populations over a very short time period.

This threat may have been particularly relevant to the historic Threehorn Wartyback population in the Detroit River. Zebra Mussel are not only a threat for lacustrine freshwater mussel populations but do pose a threat to riverine populations should they become established in reservoirs. Impoundments behind reservoirs act to increase water retention times, allowing time for Zebra Mussel veligers to settle and act as a seed population. Infestation may occur if water retention time is greater than the life span of the larval stage of the Zebra Mussel (G. Mackie, University of Guelph Emeritus, pers. comm.). Zebra Mussel have already been reported in two reservoirs on the Thames River (Upper Thames River Conservation Authority 2003), and have been noted to occur throughout the lower Thames River from Fanshawe Reservoir to the mouth of the river (Morris and Edwards 2007). Live Zebra Mussel have also been observed during field sampling in the Thames River, where live Threehorn Wartyback were observed (Morris and Edwards 2007; DFO, unpubl. data). Zebra Mussel are also known to occur in the Grand River from the mouth to Dunnville Dam, which encompasses a large proportion of the Threehorn Wartyback population in that system.

Round Goby (*Neogobius melanostomus*), another invasive species that is now prolific throughout the lower Great Lakes and tributaries, may also be negatively affecting Threehorn Wartyback. Round Goby have been shown to predate on Zebra Mussel (Ghedotti et al. 1995; Ray and Corkum 1997) but it is unknown whether Round Goby are currently utilizing native unionid mussels as a food source. Round Goby gape size limitations may be restricting Round Goby predation on Threehorn Wartyback. Ray and Corkum (1997) indicated that only large, adult Round Goby (8.5-10.3 cm in length) had the ability to prey on Zebra Mussel with shell sizes ranging from 10-12.9 mm, while smaller Round Goby size classes could only predate on smaller mussel size classes (<10 mm shell length). Therefore, gape size limitations (maximum 12.9 mm; Ray and Corkum 1997) would restrict the ability of Round Goby to prey on adult Threehorn Wartyback, while juvenile Threehorn Wartyback may remain susceptible to predation. In addition to negatively affecting Threehorn Wartyback through predation, Round Goby may also be inhibiting unionid recruitment by acting as a host. Tremblay (2012) tested the infestation and metamorphosis rates of four mussel species at risk and compared them to rates obtained from known host fish in a laboratory setting. It was concluded that Round Goby serves more as a sink for glochidia than as a host, and may be negatively affecting freshwater mussels by disrupting their reproductive cycle (Tremblay 2012). In addition to the direct affect that Round Goby may have on Threehorn Wartyback, Round Goby may be negatively affecting Threehorn Wartyback through competition with, and predation of host fishes (see threats section on Host fishes).

The feeding behaviour of Common Carp is known to have serious negative impacts on aquatic systems by uprooting aquatic vegetation and increasing turbidity levels (Lougheed et al. 1998; Lougheed et al. 2004). This feeding behaviour, known to cause significant alterations to native habitats, may impact Threehorn Wartyback. In addition, Common Carp has been shown to be the cause of bottom sediment re-suspension, increasing nutrient levels, leading to hypereutrophic conditions (Mayer et al. 1999). A study at Point Pelee National Park (Sanctuary Pond) was completed in 1994 to determine the cause of elevated nutrient concentrations leading to prolific algal growth (Mayer et al. 1999). It was determined that organic matter

decomposition was an important mechanism leading to high concentrations of nutrients, and that re-suspension of bottom sediment, primarily by Common Carp foraging behaviour, were most likely responsible for the hypereutrophic conditions (Mayer et al. 1999). The negative effects of Common Carp may be particularly relevant to Thames River and the lower Grand River where they are known to be prolific.

To facilitate the threat level assessment, and to provide context on which invasive species is being considered for each Threehorn Wartyback population, Table 6 was created to highlight the current known distribution of dreissenid mussels, Round Goby and Common Carp.

Table 6. Current known distribution of dreissenid mussels, Round Goby and Common Carp in areas where live Threehorn Wartyback have been recorded to occur. It should be noted that the presence of the invasive species is only being considered within the known range of Threehorn Wartyback (see Current Status for additional information).

	Invasive Species		
	Dreissenid mussels	**Round Goby**	**Common Carp**
Sydenham River		X	X
Thames River	X	X	X
Grand River	X	X	X

Habitat loss and alteration

Physical loss of freshwater mussel habitat can occur as a result of many activities, such as dredging, infilling, construction of impoundments, marinas and docks, and channelization. There is no quantitative information available related to the number of freshwater mussel affected by these human activities; however, it is conceivable that removal or alteration of preferred habitat could have a direct negative effect on the recovery or survival of freshwater mussels.

Altered flow regimes

The presence of impoundments and dams on freshwater streams and rivers has been shown to negatively affect mussel communities (Vaughn and Taylor 1999; Parmalee and Polhemus 2004). Impoundments typically result in siltation, stagnation, loss of shallow water habitat, pollutant accumulation and water of poor quality due to high nutrient concentrations, while dams alter flow and can affect the natural thermal profile (Bogan 1993; Vaughn and Taylor 1999; Watters 2000). In addition, poor management of water control structures can potentially dewater areas, leading to unsuitable habitat for mussels as the bottom of the watercourse may become exposed. Dams can also cause sediment retention upstream and scouring downstream. Increased pressures from urbanization can include increased water taking from rivers as well as storm water management that greatly alter flow regimes surrounding urbanized centers. Man-made alterations to the environment have also been detrimental to mussel communities (Watters 2000). The Grand River is a highly altered system with a number of water control structures (e.g., dams and weirs). The most significant dam found within the known range of Threehorn Wartyback is the Dunnville Dam, as Threehorn Wartyback shells or live individuals have been observed both upstream and downstream of the dam.

Host fishes

Due to the obligate glochidial encystement stage, Threehorn Wartyback is also directly affected by host fish abundance and indirectly by the threats affecting the host fish. The distribution of many freshwater mussel species can be limited by the distribution of its host fish. If host fish populations decline, recruitment will not occur, and the mussel species may become functionally extinct (Bogan 1993). Movement is minimal in adult freshwater mussels and therefore mussels rely on host fish for dispersal into new habitats. Common Shiner and Longnose Dace are the putative host fishes for Threehorn Wartyback in Canada (see the Habitat Requirements section of this report).

Invasive species

Although suspected to have little direct negative effect on Threehorn Wartyback (Poos et al. 2010), Round Goby may be negatively effecting Longnose Dace, a benthic species, and one of the putative host fishes of Threehorn Wartyback. Thomas and Haas (2004) studied the decline in abundance of three native benthic species [Johnny Darter (*Etheostoma nigrum*), Logperch (*Percina caprodes*) and Trout-Perch (*Percopsis omiscomaycus*)] over a six year study, and attributed this decline to competitive interactions with Round Goby. Although, Longnose Dace was not included in this study, as it focused on lacustrine species, it is conceivable that Round Goby may be competing with Longnose Dace. Currently, there is no evidence to suggest that Round Goby is negatively affecting Common Shiner, the alternate putative fish host for Threehorn Wartyback in Canada. Alternatively, it is possible that Round Goby may be affecting Threehorn Wartyback directly by feeding on Threehorn Wartyback conglutinate. However, there is no evidence of this occurring in the scientific literature.

Barriers to movement

Threats to host fishes include barriers to movement such as impoundments and dams which limits the dispersal ability of the host fishes. For example, improvements of the Grand River mussel community have been linked to the addition of fish ladders in this system, allowing for mussel dispersal via the host fishes (Metcalfe-Smith et al. 2000). Fish passage at the Dunnville Dam (the most significant dam within the known Threehorn Wartyback distribution) was thought to have improved in 1994 with the creation of a Denil-type fishway, allowing for movement of non-jumping fish across the Dunnville Dam (Grand River Conservation Authority 2013).

It has been reported that the fishway is currently damaged and lack of maintenance has resulted in a system that is filled with debris (N. Ward, Grand River Conservation Authority, pers. comm.). The fishway is not working effectively, fish movement is likely limited, and it is unlikely that non-jumping species could access the upper river.

Climate change

Through discussions on the effects of climate change on aquatic species, impacts such as decreases in water levels, increases in water and air temperatures, increases in the frequency of extreme weather events, and emergence of diseases have been highlighted, all of which may negatively impact native freshwater mussels (Lemmen and Warren 2004). Although the various climate models provide differing projections on the long-term effects of climate change, many scenarios indicate that there will be a decrease in average annual water levels and changes in the seasonal hydrograph (Lofgren and Hunter 2011). Large water level fluctuations may result in the creation of inhabitable environments. Since the effects of climate change on freshwater mussels are speculative, it is difficult to determine the likelihood and impact of this threat on each population; therefore, the threat of climate change is not included in the following population-specific Threat Level assessment.

THREAT LEVEL ASSESSMENT

Each threat was ranked in terms of the Threat Likelihood and Threat Impact for all locations where it is believed that a Threehorn Wartyback population currently exists. The criteria used to determine whether a site would be included in the Population Status assessment was also applied to the Threat Level assessment, with the exception of Rondeau Bay, which was excluded from the Threat Level assessment based on the rationale provide previously (refer to Population Categorization section of this document).

The Threat Likelihood was assigned as Known, Likely, Unlikely, or Unknown, and the Threat Impact was assigned as High, Medium, Low, or Unknown (Table 7-10). Threat Likelihood was classified for the extent of the known distribution for each population. If location-specific information was not available, knowledge of the threat throughout the watershed was applied. Location-specific information was used to categorize the Threat Impact for each location. If location-specific information was not available, the highest Threat Impact ranking for all known populations was used. Certainty of the Threat Impact was classified and is based on: 1= causative studies; 2=correlative studies; and, 3=expert opinion. The Threat Likelihood and Threat Impact for each location were subsequently combined in the Threat Level matrix (Table 9) resulting in the final Threat Level assessment for each location (Table 10).

Table 7. Definition of terms used to describe Threat Likelihood and Threat Impact.

Term	Definition
Threat Likelihood	
Known (K)	This threat has been recorded to occur at site X.
Likely (L)	There is a > 50% chance of this threat occurring at site X.
Unlikely (U)	There is a < 50% chance of this threat occurring at site X.
Unknown (UK)	There are no data or prior knowledge of this threat occurring at site X.
Threat Impact	
High (H)	If threat was to occur, it would jeopardize the survival or recovery of this population.
Medium (M)	If threat was to occur, it would likely jeopardize the survival or recovery of this population.
Low (L)	If threat was to occur, it would be unlikely to jeopardize the survival or recovery of this population.
Unknown (UK)	There is no prior knowledge, literature or data to guide the assessment of the impact if it were to occur.

Table 8. Threat Likelihood and Threat Impact of each Threehorn Wartyback population in Canada. The Threat Likelihood was assigned as Known (K), Likely (L), Unlikely (U), or Unknown (UK), and the Threat Impact was assigned as High (H), Medium (M), Low (L), or Unknown (UK). Certainty is associated with Threat Impact (TI) and is based on the best available data (1= causative studies; 2=correlative studies; and 3=expert opinion). References (Ref) are provided.

	Sydenham River				Thames River			
	TLH	TI	C	Ref	TLH	TI	C	Ref
Contaminants and toxic substances	K	H	3	9,11	K	H	3	9,10,12
Nutrient loading	K	M	3	11	K	M	3	12
Turbidity	K	M	3	4,13	K	UK	3	5
Sediment loading	K	M	3	4	K	M	3	5
Invasive species	K	L	2	1,2,3	K	H	2	1,2,3
Habitat removal and alteration	K	H	3	12	L	H	3	12
Altered flow regimes	U	M	3	13	U	M	3	12
Host fish (invasive species)	K	UK	3	2	K	UK	3	2
Host fish (barriers to movement)	U	H	3	13	U	H	3	13

	Grand River			
	TLH	TI	C	Ref
Contaminants and toxic substances	K	H	3	6,8,9
Nutrient loading	K	H	3	8,12,14
Turbidity	K	UK	3	6
Sediment loading	K	M	3	8
Invasive species	K	H	2	1,3
Habitat removal and alteration	K	H	3	12
Altered flow regimes	K	M	3	12
Host fish (invasive species)	K	UK	3	2
Host fish (barriers to movement)	K	H	3	7

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1. Ontario's Invading Species Awareness Program
2. DFO, unpubl. data
3. Therriault et al. (2013)
4. Dextrase et al. (2003)
5. Taylor et al. (2004)
6. Water Quality Branch (1989a)
7. N. Ward, Grand River Conservation Authority, pers. comm.
8. MacDougall and Ryan (2012)
9. Dove et al. (2002)
10. Water Quality Branch (1989b)
11. Bouvier and Morris (2010)
12. COSEWIC (2006a)
13. Threehorn Wartyback Recovery Potential Assessment participants; Meeting held 10 December 2013 in Burlington, Ontario
14. MacDougall and Ryan (2012)

Table 9. The Threat Level Matrix combines the Threat Likelihood and Threat Impact rankings to establish the Threat Level for each Threehorn Wartyback population in Canada. The resulting Threat Level has been categorized as Poor, Fair, Good, or Unknown.

		Threat Impact			
		Low (L)	**Medium (M)**	**High (H)**	**Unknown (UK)**
Threat Likelihood	**Known (K)**	Low	Medium	High	Unknown
	Likely (L)	Low	Medium	High	Unknown
	Unlikely (U)	Low	Low	Medium	Unknown
	Unknown (UK)	Unknown	Unknown	Unknown	Unknown

Table 10. Threat Level for Threehorn Wartyback populations, resulting from an analysis of both the Threat Likelihood and Threat Impact. The number in brackets refers to the level of certainty assigned to each Threat Level, which relates to the level of certainty associated with Threat Impact. Certainty has been classified as: 1= causative studies; 2=correlative studies; and 3=expert opinion.

Threat	Sydenham River	Thames River	Grand River
Contaminants and toxic substances	High (3)	High (3)	High (3)
Nutrient loading	Medium (3)	Medium (3)	High (3)
Turbidity	Medium (3)	Unknown (3)	Unknown (3)
Sediment loading	Medium (3)	Medium (3)	Medium (3)
Invasive species	Low (2)	High (2)	High (2)
Habitat removal and alteration	High (3)	High (3)	High (3)
Altered flow regimes	Low (3)	Low (3)	Medium (3)
Host fish (invasive species)	Unknown (3)	Unknown (3)	Unknown (3)

MITIGATIONS AND ALTERNATIVES

Threats to species survival and recovery can be reduced by implementing mitigation measures to reduce or eliminate potential harmful effects that could result from works or undertakings associated with projects, or activities in Threehorn Wartyback habitat. Threehorn Wartyback has been assessed as Threatened by COSEWIC and is not currently listed nor protected under the *Endangered Species Act*, 2007.

Within Threehorn Wartyback habitat, a variety of works, undertakings, and activities have occurred in the past few years with project types including: water crossings (e.g., bridge maintenance); shoreline and streambank works (e.g., stabilization); instream works (e.g., channel maintenance): the placement or removal of structures in water. A review has been completed summarizing the types of work, activity, or projects that have been undertaken in habitat known to be occupied by Threehorn Wartyback (Table 11). The DFO Program Activity Tracking for Habitat (PATH) database, as well as summary reports of fish habitat projects reviewed by partner agencies (e.g., conservation authorities), have been reviewed to estimate the number of projects that have occurred during the three-year period, 2010-2012. Only 18 projects were identified in Threehorn Wartyback habitat, but likely do not represent a comprehensive list of activities that have occurred in these areas (Table 11). Some projects occurring in proximity but not in the area of habitat may also have impacts, but were not included. Some projects may not have been reported to partner agencies or DFO if they occurred under conditions of an Operational Statement. It was noted that seven were completed under conditions of Operational Statements primarily for bridge maintenance.

Only one project to replace the Cayuga bridge was authorized under the *Fisheries Act* and permitted under the *SARA* since a mussel relocation for other SARA species was required. No Threehorn Wartyback were found during the relocation. The remaining projects were also deemed low risk to fish and fish habitat and were addressed through letters of advice with standard mitigation. Without appropriate mitigation, projects or activities occurring adjacent or close to these areas could have impacted Threehorn Wartyback (e.g., increased turbidity or sedimentation from upstream channel works).

Table 11. Summary of works, projects and activities that have occurred during the period of January 2010 to December 2012 in areas known to be occupied by Threehorn Wartyback. Threats known to be associated with these types of works, projects, and activities have been indicated by a checkmark. The number of works, projects, and activities associated with each Threehorn Wartyback population, as determined from the project assessment analysis, has been provided. Applicable Pathways of Effects have been indicated for each threat associated with a work, project or activity (1 - Vegetation clearing; 2 – Grading; 3 –Excavation; 4 – Use of explosives; 5 – Use of industrial equipment; 6 – Cleaning or maintenance of bridges or other structures; 7 – Riparian planting; 8 – Streamside livestock grazing; 9 – Marine seismic surveys; 10 – Placement of material or structures in water; 11 – Dredging; 12 – Water extraction; 13 – Organic debris management; 14 – Wastewater management; 15 – Addition or removal of aquatic vegetation; 16 – Change in timing, duration and frequency of flow; 17 – Fish passage issues; 18 – Structure removal; 19 – Placement of marine finfish aquaculture site).

Work/Project/Activity	**Threats** (associated with work/project/activity)						**Watercourse / Waterbody** (number of works/projects/activities between 2010-2012)		
	Contaminants and toxic substances	**Nutrient loading**	**Turbidity and sediment loading**	**Habitat removal and alteration**	**Altered flow regimes**	**Host fish (barriers to movement)**	**Sydenham River**	**Thames River**	**Grand River**
Applicable pathways of effects for threat mitigation and project alternatives	1,4,5,6, 7,11,12,13, 14, 15,16,18	1,4,7,8, 11,12, 13,14, 15,16	1,2,3 4,5, 6,7,8,10, 11,12,13, 15,16,18	1,2,3,4,5,7,8, 10,11,13,14, 15,16,18	10,16, 17	10,16, 17			
Water crossings (bridges, culverts, open cut crossings)	✓		✓	✓	✓	✓	4	2	3
Shoreline, streambank work (stabilization, infilling, retaining walls, riparian vegetation management)	✓		✓	✓	✓			1	1
Dams, barriers, structures in water (maintenance, modification, hydro retrofits)			✓	✓	✓	✓			
Instream works (channel maintenance, restoration, modifications, realignments, dredging, aquatic vegetation removal)	✓	✓	✓	✓	✓		2		1
Water management (stormwater management, water withdrawal)	✓	✓	✓		✓				
Structures in water (boat launches, docks, effluent outfalls, water intakes)	✓	✓	✓	✓	✓			3	1

The most frequent project type (nine of 18) was for water crossings which includes directional drilling for piping. Based on the assumption that historic and anticipated development pressures are likely to be similar, it is expected that similar types of projects will likely occur in or near Threehorn Wartyback habitat in the future. The primary project proponents were local municipalities.

As indicated in the Threat Analysis, numerous threats affecting Threehorn Wartyback populations are habitat-related threats that have been linked to the Pathways of Effects developed by DFO Fish Habitat Management (FHM) (Table 11). DFO FHM has developed guidance on mitigation measures for 19 Pathways of Effects for the protection of aquatic species at risk in the Central and Arctic Region (Coker et al. 2010). This guidance should be referred to when considering mitigation and alternative strategies for habitat-related threats. At the present time, we are unaware of mitigation that would apply beyond what is included in the Pathways of Effects.

Non-habitat related activities require additional discussion as these activities are not considered in the guidance on mitigation measures (Coker et al. 2010). Mitigation and alternative measures to invasive species and host fishes, as it relates to Threehorn Wartyback, are proposed.

Invasive species

As discussed in the **THREATS** section, aquatic invasive species (e.g., dreissenid mussels, Round Goby and Common Carp) introduction and establishment may have a negative effect on Threehorn Wartyback populations. Mitigation and alternatives should not only be considered for current established invasive species but species that may invade in the future.

Mitigation

- Evaluate the likelihood that a waterbody will be invaded by an invasive species.
- Monitor watersheds for invasive species that may negatively affect Threehorn Wartyback populations directly, or negatively affect Threehorn Wartyback preferred habitat.
- Develop a plan to address potential risks, impacts, and proposed actions if monitoring detects the arrival or establishment of an invasive species.
- Introduce a public awareness campaign on proper boat cleaning methods when transferring boats from an infested waterway, and on the proper identification of native and invasive freshwater mussels. The public awareness campaign could include an educational fact sheet to better educate the public on native and invasive species.
- Encourage the use of existing invasive species reporting systems.
- Restrict the use of boats in areas particularly susceptible to Zebra Mussel introduction and infestation.

Alternatives

- Unauthorized
    - None.
- Authorized
    - Use only native species.
    - Follow the National Code on Introductions and Transfers of Aquatic Organisms for all aquatic organism introductions (DFO 2003).

Host Fish

As discussed in the **THREATS** section, decreases in the number of individual host fish or decreases in the area of overlap between host fish and freshwater mussel may decrease the likelihood that a fish-mussel encounter will occur.

Mitigation

- Once the functional host fishes are confirmed for Threehorn Wartyback in Canadian waters, and if populations appear to be decreasing, a management plan for the appropriate host fish should be implemented. This would increase the host's survival, increasing the number of hosts available, creating a healthy host population and subsequently increasing the likelihood that the host fish would encounter a gravid freshwater mussel.
- The removal of barriers to host fish movement should be considered to allow increased host fish access to areas known to be inhabited by Threehorn Wartyback, if barriers to movement is deemed a limiting factor in the survival and recovery of Threehorn Wartyback.

Alternatives

- Enhance fish passage where barriers may be impeding the movement of host fishes.
- Artificially propagate host fish species where the abundance of host fish species is determined to be a limiting factor in the recovery or survival of Threehorn Wartyback.
- In areas where host fish species is abundant, artificial propagation of Threehorn Wartyback to enhance current populations should be explored.

SOURCES OF UNCERTAINTY

Despite concerted efforts to increase our knowledge of Threehorn Wartyback in Canada, there are still a number of key sources of uncertainty for this species related to population distribution, structure, habitat preferences and to the factors limiting their existence.

There is a need for a continuation of quantitative sampling of Threehorn Wartyback in areas where it is known to occur to determine population size, current trajectory, and trends over time. There is also a need for additional targeted sampling in the Grand River, as very few current records of live individuals exist for this system. Exploratory sampling should be completed in systems with habitat characteristics similar to those areas where Threehorn Wartyback is known to occur to determine the extent of their distribution. Sampling of rarely sampled deep water habitat should also occur to determine if Threehorn Wartyback are occupying these areas. In addition, supplementary sampling is necessary for all populations that were assigned a low certainty in completing the population status assessment. As is now common practice, shell length of all live individuals should be recorded to gain information on population structure and to understand recruitment within each population. These baseline data are required to monitor Threehorn Wartyback distribution and population trends as well as the success of any recovery measures implemented.

Additional studies on habitat requirements are imperative to determine critical habitat for all Threehorn Wartyback life stages. Laboratory experiments, and if feasible field experiments, should be completed to determine the functional host fish of Threehorn Wartyback in Canada. Currently, putative host fish species are inferred from infestation experiments in the United States. Infestation experiments, using samples from Canadian populations, should be

completed to verify the usage of Common Shiner and Longnose Dace as host fishes for Threehorn Wartyback. Sampling of putative host fish should be completed in areas known to be inhabited by Threehorn Wartyback, during which the gills should be inspected and sampled for Threehorn Wartyback glochidia. This may aid in determining the host fish from a natural infestation. Once host fish species have been confirmed, additional investigations to determine the glochidial carrying capacity, as well as the relationship between mussel attachment probability and host-mussel density should be completed.

The largest barrier preventing accurate population modelling of Threehorn Wartyback is a lack of sufficient length- or age-frequency data. Small sample size is often a challenge when studying rare species and existing sample sizes are insufficient to perform catch curve analyses for estimation of adult survival. In addition, very little is known about glochidial attachment and survival of Unionids in general.

Numerous threats have been identified for Threehorn Wartyback populations in Canada, although the direct impact that these threats may have is currently unknown. There is a need for more quantitative studies to evaluate the direct impact of each threat on Threehorn Wartyback populations with greater certainty. In the literature, the threat impacts are generally discussed at a broad level (i.e., mussel assemblage level). It is important to further our knowledge on threat likelihood and impact at the species level. Research is needed to determine the effect of contaminants and toxic substances on Threehorn Wartyback, as these pollutants are known to occur in areas where Threehorn Wartyback is currently found. This type of research would provide insight on the factors currently limiting Threehorn Wartyback populations. Thresholds for other water quality parameters (e.g., nutrients, turbidity) should also be investigated.

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